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EFFECTS OF ORAL ADMINISTRATION OF ETHANOLIC LEAF EXTRACT OF *MUCANA PURIENS* OBTAINED FROM AFIKPO ON SERUM LIVER BIOMARKERS AND SEX HORMONES IN ALBINO WISTAR RATS

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ABSTRACT

The effects of oral administration of ethanolic leaf extract of Mucana puriens obtained from Afikpo, Ebonyi State, Nigeria for 28 days on some serum biochemical parameters and electrolytes of albino wistar rats were investigated using standard protocols. Thirty-two (32) albino rats comprising 16 males and 16 females were used for the study. The rats were divided into four groups namely A, B, C and D. Each group was made up of 4 male and 4 female rats separated but given similar treatment. Group A served as the control and were treated with normal saline (0.4ml/100g body weight). Each of groups B, C, and D were administered through oral intubation 250 mg/kg body weight, 500 mg/kg body weight and 750 mg/kg body weight of extract respectively. The result of acute toxicity test obtained showed a lethal dose (LD_{50}) of greater than 5000mg/kg body weight of the extract. Results indicated that administered doses of 250, 500 and 750mg/kg body weight of the leaf extract showed dose dependent significant ($P < 0.05$) decrease in serum Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline Phosphatase (ALP) when compared to the control. Also the results showed significant ($P < 0.05$) dose dependent increase in progesterone, follicle stimulating hormone and luteinizing

hormone level for both the male or female rats when compared to control. Estradiol, prolactin and estrogen levels increased significantly at P < 0.05 in female rats only while testosterone levels increseased significantly at P < 0.05 in male rats only. The implications of these results were discussed.

Key words: *Mucana puriens*, Liver biomarker, Sex hormones, Fertility, Afikpo

Introduction

The increasing use of plants and Plant-derived chemicals (products) in ethno-medicine as valuable sources of therapeutic agents is the mainstay in the treatment of various diseases and preservation of human health worldwide (Sofowora, 1993; Ebomoyi *et al*., 2004). Some of these plants are known to influence endocrine activities in both humans and animals and as such, have received a great deal of attention due to their possible beneficial as well as adverse effects (Gamache and Acworth, 1998). Several plant extracts are known to possess regulatory activity on the reproductive functions. The reproductive functions and characteristics in both male and female organisms are known to be regulated by sex hormone (Granner, 2000; Chartterjee and Shinde, 2002). Sex hormones such as testosterone, follicle-stimulating hormones and luteinizing hormones among others are very important in human reproduction and development since they are involved in the production and maturation of sex cells and promotion of secondary sexual characteristics (Rahman, 2001). Reports have shown that luteinizing hormones act on the Leydig cells of the testes and that this is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis (Nielsen *et al*., 2001). Also, anti-androgens exert effect through their action on the hypothalamus-pituitary-gonadal axis or direct hormonal effect on reproductive organs and this leads to the inhibition of spermatogenesis or ovarian steroidogenesis resulting to infertility (Shibeshil *et al*., 2006). Therefore, measurement of serum sex hormones profile is a very useful bio-indicator in assessing the reproductive integrity in both animals and humans. For example, reductions in levels of testosterone and follicle stimulating hormone (FSH) have been implicated to be responsible for suppressed potency, hormonal imbalance and sexual dysfunctions in males (Uboh et al., 2010; Gelain *et al*., 2005; Greenspan and Strewler, 1997) while serum estradiol measurement has been

reported as a valuable index in evaluating a variety of menstrual dysfunctions in females (Jenner *et al*., 1982). Thus, suppression or improvement of reproductive functions may be implicated in reduced or increased serum sex hormone profile respectively.

In Africa and most developing countries of the world where folkloric medicine is the mainstay of the populace, decoctions from plants' parts are commonly employed to treat a wide range of reproductive disorders. Most of the plants' extracts are particularly believed to improve erection and potency in males and as well improve female fertility through improved ovarian steroidogenesis. One of these plants is *Mucuna pruriens*. The plant is an annual, climbing shrub with long vines that can reach over 15 m in length (Katzenschlager *et al*., 2004; Akpanabiatu *et al*., 2005). *Mucuna pruriens* is underutilized plant found in Africa, India and Caribbean and it is unpopular due to the extreme itchiness it produce on contact, particularly with the young foliage and the seed pods. The plant parts have been reported to exhibit significant aphrodisiac, antispasmodic, anticataleptic, antiepileptic, anti-diabetic, antimicrobial, anti-inflammatory, pain-relieving and fever-reducing activities from various clinical researches with animal models (Amin *et al*., 1996; Hussain and Manyam, 1997; Manyam *et al*., 2004; Sathiyanaryanan and Arulmozhi, 2007; Majekodunmi *et al.,* 2011; Champasingh *et al.,* 2011; Lampariello *et al*., 2012). The leaves are consumed for their nutritional value and its extracts are also used in folk medicine as a therapy for various diseases such as infertility, diabetes, arthritis, dysentery, infertility, obesity and cardiovascular disorders, blood booster and for maintenance of homeostasis (Nadkaru, 2001; Bishop *et al*., 2010; Ram and Mohammad, 2011).

The diverse and increased use of *Mucuna pruriens* parts and products in laboratory evidenced treatment of numerous ailment in animal models and its folkloric medicinal uses in humans without documented evidence to support the practice calls for the need to provide information on the safety or toxicity risk of the plants' parts. This study therefore assessed the effects of leaf extracts on serum liver biomarker and sex hormonal profile in male and female albino wistar rats, with the aim of investigating the safety, validity or otherwise of the use of the leaf extract in reproductive disorders in folkloric medicine.

MATERIALS AND METHODS

Plant material

Mucuna pruriens sample was collected from Akpoha in the month of May 2013 and was authenticated with the help of Mrs Stella Eberechukwu Obasi of the Plants and Environmental Biology Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana where a voucher specimen has been deposited.

Extraction

Fresh leaves of *Mucuna pruriens* (500g) were air died for seven days under atmospheric conditions and pulverized to fine powder using milling grinder (Thomas Wiley Model 4). The pulverized material was macerated in 70% ethanol to give an extract that was filtered. The filtrate was concentrated using a rotary evaporator to yield viscous slurry with a percentage recovery of 9.4%.

Animal Treatment

Thirty-two (32) albino rats of both sexes comprising 16 males and 16 females and weighing 150-200 grams were used for the study. The animals were housed in the Animal House, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, Nigeria. The animals were fed on standard feeds (Grower pellets of Royal feeds, Enugu, Nigeria) and allowed access to water *ad libitum*. The "Principle of laboratory animal care " (NIH publication No 85-23)'' guideline and procedures were followed in this study (NIH publication reserved 1985).

The animals were randomized into experimental and control groups and were kept in polypropylene cages. The rats were divided into four groups namely A, B, C and D. Each group was made up of 4 male and 4 female rats separated but given similar treatment. Group A served as the control and were treated with normal saline (0.4ml/100g body weight). Each of groups B, C, and D were administered through oral intubation 250 mg/kg body weight, 500 mg/kg body weight and 750 mg/kg body weight of the plants extract respectively. All administrations were orally by gastric intubation. After twenty eight days of treatment, the animals in each group were sacrificed by chloroform anesthesia. Blood was collected by cardiac puncture and allowed to clot, centrifuged at 3000 rpm for 15mins and the serum aspirated. The separated serum samples were stored in the refrigerator until required for the liver biomarker and hormonal assay. All assays were done within 24 hours of the sample collection.

Chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade.

Acute Toxicity Test

This was done using the Lorke's method (Lorke, 1983). A total of 20 albino wistar rats weighing between 150 –200g were fasted overnight although with access to tap water. They were divided into five groups of four rats each comprising two males and two females. Four groups were given different doses of the extract in the following order: 200, 500, 2000, 5000mg/kg body weight of the rats while the fifth group (control) received only normal saline. The rats were observed for 12 hours for any lethality or signs of overt toxicity.

Serum Liver Biomarker and Hormonal Assay

Alkaline phosphatase (ALP) activity was determined by the method of Ahamed and King (1959) while the activities of aspartate and alanine aminotransferases (AST and ALT respectively) were determined by the method of Reitman and Frankel (1957).

The serum samples were assayed for progesterone, estradiol, follicle stimulating hormone (FSH), prolactin, testosterone, Leutinizing hormone (LH) and estrogen using enzymes immunoassay methods. The respective immunoassay reagent kits were obtained from Diagnostic Automation Inc., 23961 Craftman Road, Suite E/E, Calabasas, CA 91302. Microplate reader (Dialab Instruments Ltd.) was used in taking the absorbance. Calculations of the concentrations of hormones were made according to the method given in the kits manual.

RESULTS

The results of the effects of graded doses of ethanolic leaf extract of *Mucana puriens* orally administered to Albino wistar rats on liver biomarkers are shown in Figures 1 to 3. The results indicated a dose dependent significant decrease at $P < 0.05$ in serum alanine transaminase (ALT), serum aspartate transaminase (AST) and serum alkaline phosphatase (ALP) when compared to the control with 250mg/kg and 750 mg/kg body weight producing the lowest and highest level of serum AST, ALT and ALP.

The results of effects of ethanolic extract of *Mucuna pruriens* leaf on reproductive hormones of albino wistar rats are shown in Table 1. The results show significant ($P < 0.05$) dose dependent increase in progesterone, follicle stimulating hormone and luteinizing hormone level for both the male or female rats when compared to control. Testosterone levels increseased significantly at $P < 0.05$ in a dose dependent manner when compared to control in male rats while they decreased

significantly at $P < 0.05$ in female rats (Table 1). The results also showed that there was a dose dependent significant increase at $P < 0.05$ in the level of estradiol, prolactin and estrogen in female rats and a dose dependent significant decrease at $P < 0.05$ of the level of these hormones in the male rats (Table 1).

DISCUSSION

Herbal medicine is gaining popularity in developing countries due to its broad spectrum nature in tackling a myriad of diseases (Farnsworth, 1989; Gbile and Adesina, 1989). Reports have shown that greater percentage of the world's population still depend mainly on traditional medicine which involve mainly the use of plant extracts and products (Akarele, 1993; Saggu *et al*., 2007). Aminotransferases are useful ''biomarker'' enzymes of liver cytolysis (Amresh *et al*., 2008; Yakubu *et al*., 2005). These enzymes occupy a central position in the metabolism of amino acids as they help to retain amino groups (to form a new one) during the degradation of amino acids. The dose dependent decrease in serum alanine transaminase (ALT), serum aspartate transaminase (AST) activities observed in this study (Figures 1-2) could be explained partly by a decrease in the functional activity of the liver resulting in reduction in the induction of these enzymes without consequential effects on the metabolism and regulation of their amino acids substrates. The decrease in serum aminotransferase activity may also be attributed to the fact that the enzymes did not leak out from the liver cells and as such confirm no change in membrane permeability of these cells or no change in liver integrity (Latha *et al*., 1998). Thus, the results of this study showed that this plant extract is safe and non-toxic to the liver.

Alkaline phosphatase (ALP) is a ''biomarker'' enzyme clinically used to assess damage to the plasma membrane and endoplasmic reticulum (Shahjahan *et al*., 2004) and as such, it is often used to assess the integrity of the plasma membrane (Akanji *et al*., 1993). The results of the study indicated a dose dependent decrease in serum ALP activity (Figure 3) and this implies partly that the enzyme molecules were not induced from de novo synthesis (Yakubu *et al*., 2003). The decrease in ALP activity may not lead to indiscriminate hydrolysis of phosphate esters in the tissue. Damage to structural integrity of tissues is always reflected by an increase in some of these enzymes in the serum, probably through leakage from the altered cell membrane structure.

Therefore, the corresponding decrease in serum ALP activity confirms no damage to the plasma membrane and thus the liver integrity was not compromised (Yakubu *et al*., 2003).

Alterations or imbalances in reproductive hormones have been linked to irregularity in the reproductive function of organisms (Yakubu *et al*., 2008; Shivalingappa *et al*., 2002). Bioactive chemicals (phytochemicals) contained in plant extracts have been reported to cause these alterations in the reproductive hormones of organisms (Asuquo *et al*., 2012; Anna *et al*., 2013; Uboh *et al*., 2010). Studies on the phytochemical contents of plants have revealed many beneficial bioactive as well as toxic agents of plant extracts that can affect the regulation and normal functioning of oestrous cycle, conception and reproduction (Benie *et al*., 2003; Yakubu *et al*., 2005). Reports have shown that alkaloids and flavonoids from plants reduce plasma concentrations of luteinizing hormone, estradiol and follicle stimulating hormones (Lauritzen *et al*., 1997; Browning *et al*., 1998; Bianco *et al*., 2006). *Mucana puriens* has been reported to contain numerous phytochemicals which support their use in ethno-medicine (Tavares *et al*., 2015; Bala *et al*., 2011) and these may have contributed to the numerous effects observed in this study.

This study revealed that *M. puriens* extract increased in the level of progesterone in a dose dependent manner in both male and female albino rats (Table 1). Progesterone is produced in the ovaries, placenta, and adrenal glands and it helps to regulate the monthly menstrual cycle, prepare the body for conception and pregnancy and as well as stimulate sexual desire (Montaserti *et al*., 2007). The hormone also encourages the growth of milk-producing glands in the breast during pregnancy. The increase in the level of this hormone by the plant extract may have beneficial effects since high progesterone levels are believed to be partly responsible for relieving the symptoms of premenstrual syndrome (PMS), such as breast tenderness, feelings of bloat and mood swings and as well as balance and neutralize the powerful effects of excess estrogen (Anasti *et al*., 2004; Gocze *et al*., 1996). These results show that *M. puriens* extract may have anti-contraceptive properties. Reports have shown that estradiol stimulates the growth of the uterine lining, causing it to thicken during the preovulatory phase of the cycle and that it is directly responsible for the growth and development of reproductive organs (MacLennan *et al*., 2004; Nelson, 2004). Also, combined effects of estradiol with FSH have been shown to stimulate granulose cell proliferation during follicular development (Telefo *et al*., 1998). The increase in

the serum concentration of estradiol observed in this study (Table 1) may be attributed to a increased aromatase activity or substrate supplementation during estrogen synthesis (Hsia *et al*., 2007) because plants with estrogenic property have been reported to directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones and blocking ovulation (Brinker, 1997). Thus, such increase in estradiol levels may improve ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of the pregnancy state in females (Hadley, 2000). Our findings is in tandem with that of Ota *et al.* (1995) which demonstrated that herbal Shakuyaku (*Paeoniae radix*), Keihi (*Cinnamomi cortex*) and Botanpi (*Moutan cortex*) stimulated the aromatase activity in human granulose cells and increased estradiol secretion *in vitro*. Thus, it can be inferred that the aqueous extract of *Mucana puriens* contain biologically active phytochemicals which may be improve hormonal balance or reduce disorders such as infertility and contraception in hormone dependent organs like the ovary and mammary glands

The study showed that the plant extract increased the level of follicle stimulating hormone (Table 1) in a dose dependent manner. Reports have shown that this hormone central to mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life (Simoni and Nieschlag, 1995). Follicle stimulating hormone stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells (Nelson, 2004). The increase in the levels of FSH by the extract may induce folliculogenesis and enhance maturation of the follicle in the pre-ovulatory phase (Kumar *et al*., 1997). It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The increase in the levels of the hormone may have beneficial effect on the maturation of male secondary sex characteristics and conception in the female animals. This study is no in agreement with that reported for *Afrormosia laxiflora*, *Pterocarpus erinaceus* and *Cola nitida* stem bark whose administration decreased the release of the gonadotropins (LH and FSH) (Benie *et al.,* 2003).

Prolactin stimulates lactogenesis and helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland (Solomon *et al*., 2011). In this study, it was observed that the prolactin increased in female rats and decreased in male rats (Table 1).

Dopamine serves as the major-inhibiting factor or break on prolactin secretion (Fitzgerald and Dinan, 2008). The observed level of prolactin in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist in females and agonist in males. Reports have shown that high prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropic-releasing hormones (GnRH) which are necessary for ovulation (Fitzgerald and Dinan, 2008). Such increase in prolactin levels as observed in female rats may be used in the stimulation of lactation.

Testosterone levels were observed to increase in male rats and decrease in female rats treated with *Mucana puriens* extract (Table 1). The increase in testosterone level in the male rats may be due to increase synthesis or decreased metabolic clearance and vice versa for the females (Maneesh *et al*., 2006). The increase in the levels of FSH and LH may have stimulated the production of more testosterone in the male rats (Emanuele and Emanuele, 2001). The results show that *Mucana puriens* may have the ability to disrupt the processes necessary for the production of testosterone by Leydig cells (Udoh *et al*., 2005a:b). In males, increase of testosterone level might improve spermatogenesis and cause male fertility.

Luteinizing hormone stimulates secretion of sex steroids from the gonads. The extract in this study were observed to increase luteinizing hormones in both the felmale and male rats (Table 1). In females, ovulation of mature follicles in the ovary is induced by a large surge of LH secretion during the pre-ovulatory periods. Numerous reports have shown that LH release surge at the pre-estrous stage are responsible for ovulation (Gallo, 1981; Hashimoto *et al*., 1987). Any substance capable of inhibiting this release could provoke disruption of ovulation by decreasing the number of mature follicles or induce an oestrous cycle disruption at rest (Benie *et al.*, 2003)). Therefore, the increase in the serum LH levels may be explained by a stimulating effect of the extract on the release of LH which may trigger normal ovulation leading to normacy oestrous cycle, improved conception and normal reproduction in the females (Sulaiman *et al*., 2001a:b). These results show that *Mucana puriens* can not hinder gonadotropin release and oestrous cycle as reported for some other plants extracts (Banerje *et al*., 1999; Al-Qarawi *et al*., 2000). This study further observed a dose dependent increase in the estrogen serum level of female rats (Table 1). This increase might probably be due to the conversion of testosterone to estrogen (Carr and Blackwell, 1993; Chinoy and Padman, 1996). Overall, our findings in this study show

that *Mucana puriens* leaf extract may improve the development of male and female secondary sex characteristics.

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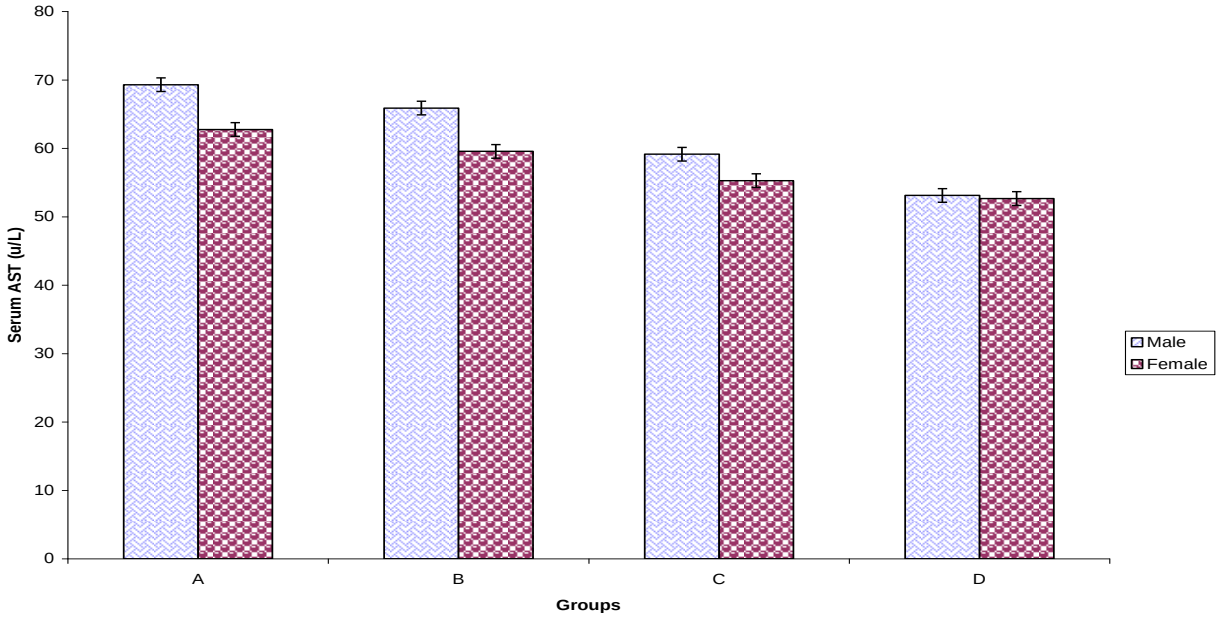

Figure 1: Effects of Ethanolic Extract of Mucana puriens leaf on Serum AST (u/L) of Albino Wistar Rats

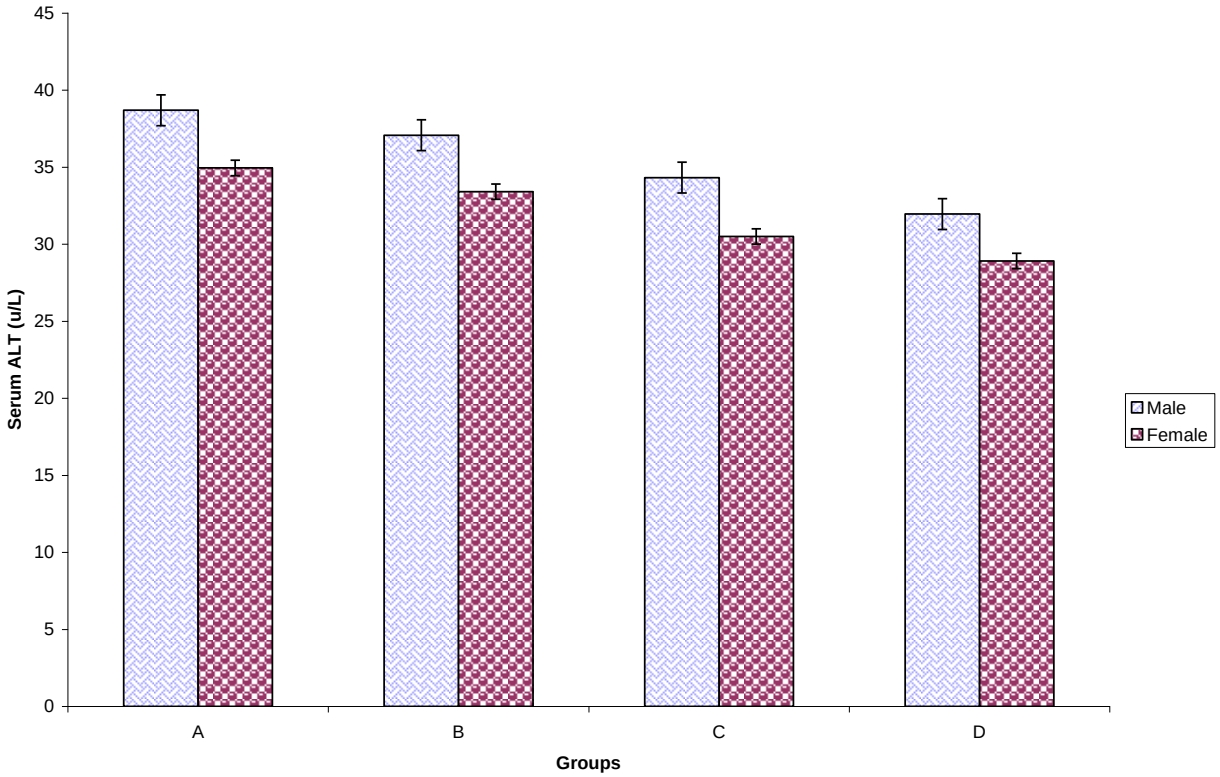

Figure 2: Effects of Ethanolic Extract of Mucana puriens leaf on Serum ALT (u/L) of Wistar Rats

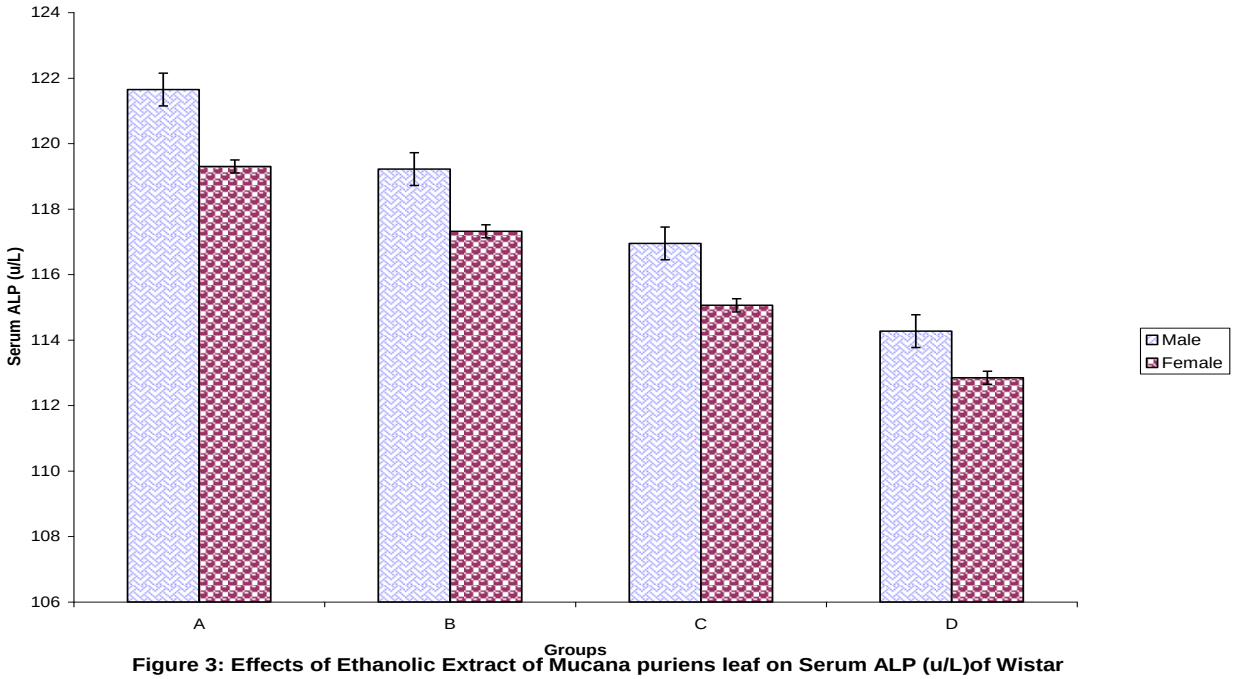

Figure 3: Effects of Ethanolic Extract of Mucana puriens leaf on Serum ALP (u/L)of Wistar Rats

Table 1: Effects of ethanolic extract of *Mucana puriens* on the reproductive hormones of Albino rats

Groups	Dose mg/kg. body wt	Progesterone (ng/dl)		Estradiol (pg/ml)		FSH (µlU/ml)		Prolactin (ng/ml)		Testosterone (ng/ml)		Luteinizing Hormone (µlU/ml)		Estrogen (pg/ml)	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
A	Control	$34.47^{a} \pm 1.03$	$38.12^{a} \pm 0.50$	$333.28^{d} \pm 0.67$	$327.67^{a} \pm 0.52$	$1.91^{a} \pm 0.05$	$2.03^{a} \pm 0.01$	$198.70^{d} \pm 0.67$	$203.77^{a} \pm 0.28$	$3.94^{a} \pm 0.07$	$3.83^{c} \pm 0.25$	$1.97^{a} \pm 0.08$	$2.02^{a} \pm 0.05$	$26.63^{a} \pm 0.06$	$26.18^{a} \pm 0.12$
B	250	$36.05^{b} \pm 0.50$	$41.77^{b} \pm 0.71$	$330.16^{c} \pm 0.25$	$329.67^{b} \pm 0.09$	$2.06^{a} \pm 0.02$	$2.11^{a} \pm 0.07$	$196.35^{c} \pm 0.13$	$204.86^{b} \pm 0.11$	$4.10^{a} \pm 0.11$	$3.70^{b} \pm 0.16$	$2.11^{a} \pm 0.03$	$2.12^{a} \pm 0.02$	$26.50^{a} \pm 0.03$	$26.34^{a} \pm 0.08$
C	500	$37.92^{b} \pm 0.67$	$45.11^{c} \pm 0.22$	$327.49^{b} \pm 0.44$	$331.14^{c} \pm 0.15$	$2.27^{b} \pm 0.05$	$2.32^{b} \pm 0.44$	$194.76^{b} \pm 0.26$	$206.11^{b} \pm 0.25$	$4.38^{b} \pm 0.06$	$3.47^{ab} \pm 0.09$	$2.34^{b} \pm 0.11$	$2.39^{b} \pm 0.13$	$26.77^{a} \pm 0.67$	$27.10^{b} \pm 0.05$
D	750	$40.68^{c} \pm 0.35$	$51.64^{d} \pm 0.25$	$323.40^{a} \pm 0.37$	$336.95^{d} \pm 0.27$	$2.44^{bc} \pm 0.25$	$2.48^{c} \pm 0.06$	$192.13^{a} \pm 0.22$	$210.54^{c} \pm 0.09$	$4.51^{c} \pm 0.05$	$3.19^{a} \pm 0.03$	$2.52^{c} \pm 0.02$	$2.55^{c} \pm 0.09$	$26.65^{a} \pm 0.05$	$27.94^{bc} \pm 0.13$